JOURNAL OF VIROLOGY, Apr. 1979, p. 267-278
0022-538X/79/04-0267/12$02.00/0

Vol. 30, No. 1

Control of Protein Synthesis in *Escherichia coli*: Control of Bacteriophage Qβ Coat Protein Synthesis After Energy Source Shift-Down

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Received for publication 19 September 1978

Escherichia coli Q13 was infected with bacteriophage Qβ and subjected to energy source shift-down (from glucose-minimal to succinate-minimal medium) 20 min after infection. Production of progeny phage was about fourfold slower in down-shifted cultures than in the cultures in glucose medium. Shift-down did not affect the rate of phage RNA replication, as measured by the rate of incorporation of [^{14}C]uracil in the presence of rifampin, with appropriate correction for the reduced entry of exogenous uracil into the UTP pool. Phage coat protein synthesis was three- to sixfold slower in down-shifted cells than in exponentially growing cells, as determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The polypeptide chain propagation rate in infected cells was unaffected by the down-shift. Thus, the reduced production of progeny phage in down-shifted cells appears to result from control of phage protein synthesis at the level of initiation of translation. The reduction in the rate of Qβ coat protein synthesis is comparable to the previously described reduction in the rate of synthesis of total *E. coli* protein and of β-galactosidase, implying that the mechanism which inhibits translation in down-shifted cells is neither messenger specific nor specific for 5′ proximal cistrons. The intracellular ATP pool size was nearly constant after shift-down; general energy depletion is thus not a predominant factor. The GTP pool, by contrast, declined by about 40%. Also, ppGpp did not accumulate in down-shifted, infected cells in the presence of rifampin, indicating that ppGpp is not the primary effector of this translational inhibition.

When growing *Escherichia coli* cells are transferred to a medium supporting a lower growth rate (shift-down), protein synthesis is inhibited (12). Westover and Jacobson (40) showed that polypeptide chain initiation is inhibited 85 to 90% while polypeptide chain propagation is unaffected by shift-down. This result explains the earlier observations of Friesen (13) and Ruscetti and Jacobson (34) that shift-down leads to a loss of polyribosomes and concomitant accumulation of 70S single ribosomes or "monosomes." The 70S monosomes from down-shifted cells were characterized by several criteria as "complexed" rather than "free" ribosomes and were found to be associated with pulse-labeled RNA (34). Jacobson and Baldassare (21) examined 70S monosomes with an electron microscope and showed that monosomes lie at or near the 5′ end of an mRNA strand. These observations are consistent with the hypothesis that in down-shifted cells there is a rate-limiting step in translation at a point subsequent to the binding of ribosomes to mRNA but prior to the formation of the first peptide bond.

Inasmuch as the degree of inhibition of β-galactosidase synthesis was approximately the same as that of total protein (40), it seemed likely that the control of translation following a shift-down is relatively nonspecific. To further study the specificity and mechanism of this control, we examined the effect of shift-down on the in vivo translation of bacteriophage Qβ RNA. Our data show that the rate of Qβ coat protein synthesis following shift-down is reduced to about the same degree as that of β-galactosidase and total protein, indicating that the control of translation is relatively nonspecific. Furthermore, Qβ coat protein, unlike β-galactosidase, is translated from an internal cistron of a polycistronic mRNA (19); hence, the control mechanism is not specific for 5′-terminal cistrons.

(A preliminary report of this work was presented at the 76th Annual Meeting of the American Society for Microbiology, Atlantic City, N. J., 2-7 May 1976.)

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MATERIALS AND METHODS

Bacteriophage and host bacterium. *E. coli* strain Q13P17 was used in this study. This strain was derived in our laboratory from the male strain Q13 (33), which requires methionine, tyrosine, and an additional organic nutrient of unknown character, has "relaxed" control of RNA synthesis, and lacks RNase I and polynucleotide phosphorylase. Strain Q13P17 was selected for its ability to grow in minimal media supplemented with only methionine and tyrosine and for its inability to grow on a solid medium containing 10 μg of rifampin per ml. Bacteriophage Qβ was obtained from K. Ippen-Ihler.

Growth and infection of bacteria. Bacteria were cultured in glucose-minimal media. A phosphate-buffered minimal medium containing 25 mM K_2HPO_4, 12.5 mM KH_2PO_4, and 40 mM NH_4Cl and supplemented with salts (1.5 mM $MgSO_4$, 0.25 mM $MnCl_2$, 0.04 mM $FeSO_4$, 0.03 mM $CaCl_2$, and 0.02 mM NaCl), 0.057 mM ascorbic acid, 10 mM glucose, and 20 μg each of methionine and tyrosine per ml was used in all experiments except where indicated otherwise. For experiments involving ^{32}P-isotope labeling, bacteria were grown in a Tris-buffered minimal medium containing 100 mM Tris-hydrochloride, pH 7.3, 20 mM NH_4Cl, and 1 mM K_2HPO_4 with the same concentrations of salts, ascorbic acid, amino acids, and glucose as in the phosphate-buffered medium. Bacteria were cultured with reciprocal shaking in a water bath at 37°C. Growth was monitored by measuring absorbance at 660 nm in a Bausch & Lomb Spectronic 20 colorimeter.

Cultures grown to a density of 3×10^8 cells/ml received 2 mM $CaCl_2$ and were infected at a multiplicity of 30 PFU/bacterium. Infection proceeded for 20 min before the shift-down was imposed. Shift-down was executed by rapidly chilling infected cells, harvesting cells by centrifugation, and resuspending them in minimal medium containing 10 mM disodium succinate as sole carbon source (34). Alternatively, cells were harvested by rapid filtration (40), but either shift-down procedure gave identical results. The centrifugation procedure took exactly 15 min but apparently did not alter the timing of the infectious cycle, as indicated by the nearly identical kinetics of phage production and RNA synthesis in infected cultures growing in glucose medium and in infected glucose-grown cultures shifted to fresh glucose medium.

Bacteriophage stocks. Cultures were grown in phosphate-buffered medium supplemented with salts and ascorbic acid as described above and with 30 mM glucose, 2 mM $CaCl_2$, 40 μg each of methionine and tyrosine per ml, and 1 mg of tryptone (Difco) per ml. At a density of about 6×10^8 cells/ml, the cultures were infected at a multiplicity of 5 PFU/bacterium. Cultures were incubated at 37°C with shaking for an additional period of 6 h. A few drops of chloroform were added, cells and debris were removed by centrifuging for 20 min at 2,500 × *g*, and solid $(NH_4)_2SO_4$ was added to the supernatant fluid to a final concentration of 260 g/liter. After overnight stirring at 4°C, the precipitated phage were collected by centrifugation, resuspended in 10 ml of phage buffer per liter of infected culture, and dialyzed at 4°C overnight against 1 liter of phage buffer. Phage buffer contained 50 mM Tris-hydrochloride, pH 7.6, 100 mM NaCl, and 10 mM EDTA. Titers of 10^{13} to 10^{14} PFU/ml were normally obtained.

Measurement of total phage production in infected cells. Cultures growing in glucose medium were down-shifted 20 min after infection as described above. At each time indicated, a sample (0.05 ml) was added to 1 ml of lysis buffer (0.12 M Tris-hydrochloride, pH 8.0, 50 mM EDTA, and 1 mg of lysozyme per ml). The mixtures were treated as described by Fromageot and Zinder (14) to determine the titers.

Radioisotope incorporation into phage RNA. Cultures were treated with 200 μg of rifampin per ml 10 min before the addition of labeled precursors. At this concentration the rate of incorporation of labeled RNA precursor into uninfected, glucose-grown cultures is less than 2% of the rate in uninfected cultures without rifampin, and it is less than 15% of the rate in infected rifampin-treated cultures. This is consistent with the observation of Fromageot and Zinder (14) that, while cellular RNA synthesis is almost completely inhibited in cultures treated with rifampin, phage-specific RNA replication is virtually unaffected. A correction for the residual incorporation into uninfected, rifampin-treated cultures was made in each experiment by a point-for-point subtraction of the radioactivity in the control (uninfected) from the radioactivity in the experimental (infected) culture.

Cells were treated with rifampin at the time of shift-down (20 min after infection) and were labeled with 1 μCi of [2-^{14}C]uracil per ml 10 min later. Methods for the assay of isotope incorporation into trichloracetic acid-insoluble material (34) and measurement of radioactivity (20) have been described previously.

Measurement of intracellular nucleotide pools. Cultures growing in Tris-buffered medium received 350 to 450 μCi of $H_3{}^{32}PO_4$ per ml and a volume of 0.1 NaOH equal to the volume of $H_3{}^{32}PO_4$ at least one generation prior to sampling to ensure equilibration of labeled phosphate with intracellular nucleotide pools (31). Cultures were infected, down-shifted, and rifampin-treated as described above.

Nucleotides were extracted with 1 M formic acid, and levels of ATP and GTP in 1-μl portions of extract were determined (6). Nucleotides were separated by thin-layer chromatography on polyethyleneimine cellulose sheets (Brinkmann Instruments, Inc., Westbury, N.Y.) with 0.85 M KH_2PO_4, pH 3.4, as the solvent. The ^{32}P-labeled materials were visualized by exposing Kodak No-screen medical X-ray film to dried chromatograms for 40 h. Exposed film was processed as recommended by Kodak. Radioactive spots on chromatograms corresponding to film darkening were located and cut out. Radioactivity was measured as described previously (20). Levels of ppGpp in 5-μl portions of extract were determined in the same way except that thin-layer sheets were run with 1.5 M KH_2PO_4, pH 3.4, as the solvent (4) and X-ray films were exposed for 18 h.

Specific activity of nucleoside triphosphate pools and determination of rate of RNA replication. The rate of phage RNA replication following shift-down was calculated by the method of Lazzarini and Dahlberg (25), slightly modified, from the rate of

[2-^{14}C]uracil incorporation into RNA described above and the relative specific activities of the intracellular nucleoside triphosphate pools. Specific activity measurements were made by use of a double-labeling technique (43). Cultures growing in minimal medium received 2.5 μCi of [5-^{3}H]uridine per ml at least one generation prior to sampling. Cultures were infected, down-shifted, rifampin-treated, and labeled with [^{14}C]uracil as described above. At the indicated times, 0.4-ml samples of culture were extracted with 1 M HCOOH (6), and 1.6 nmol of UTP was added as carrier. Nucleotides were concentrated (6) and resuspended in 20 μl of 1 M HCOOH. The levels of nucleoside triphosphates in 10-μl samples were determined in the same manner as described above for ATP except that X-ray films were exposed for 4 days.

RNase digestion of crude lysates. Cells were treated with rifampin at the time of shift-down (20 min after infection) as described above and labeled with 1 μCi of [^{14}C]uracil per ml 10 min later. Cells were incubated for 15 min before samples (0.2 ml) were removed and added to tubes containing 0.2 ml of double-strength lysis buffer (0.24 M Tris-hydrochloride, pH 8.0, 0.1 M EDTA, and 2 mg of lysozyme per ml). A drop of $CHCl_3$ was added, and the tube contents were mixed briefly, incubated at room temperature for 2 h, and refrigerated overnight. Where indicated, 50 μg of pancreatic RNase per ml was added, tubes were incubated at room temperature for 30 min, and an equal volume of cold 10% Cl_3CCOOH was added. Two drops of a 1% solution of bovine serum albumin were added to each tube, followed by 3 ml of cold 5% Cl_3CCOOH. Precipitates were collected on glass-fiber filters (Reeve-Angel) and washed with 5% Cl_3CCOOH and with ethanol. The filters were dried and radioactivity was measured (20).

Polyacrylamide gel electrophoresis and determination of rate of coat protein synthesis. Labeled proteins from rifampin-treated cells were separated by polyacrylamide slab gel electrophoresis in the presence of sodium dodecyl sulfate and visualized by autoradiography. Qβ coat protein was identified, and autoradiogram film density was used as a measure of the amount of coat protein synthesized during different time intervals after isotope addition.

Cultures were infected, down-shifted, and treated with rifampin at the time of shift-down (20 min after infection) as described above. Ten minutes later, cultures received 2 μCi of L-[^{14}C]proline per ml. At each sampling time a 0.5-ml portion of culture was treated with 50 μg of chloramphenicol in a chilled 1-ml conical tube and centrifuged in a Fisher model 59 centrifuge for 5 min at 7,000 × *g*. Each pellet was resuspended in 25 μl of 0.12 M Tris-hydrochloride, pH 8.0, containing 50 mM EDTA and 1 mg of lysozyme per ml, and one drop of chloroform was added. The tubes were agitated briefly, left at room temperature for 2 h, and refrigerated overnight. DNase (10 μg/ml) was added to each tube, tubes were incubated at room temperature for 30 min, and cells and debris were removed by centrifugation for 5 min at 7,000 × *g*. Each supernatant fluid was added to a tube containing 25 μl of 0.1 M Tris, pH 6.8, 2% sodium dodecyl sulfate, 2% β-mercaptoethanol, and 20% glycerol, and the tubes were placed in a boiling water bath for 2 min.

The procedures for slab gel preparation and electrophoresis described by Studier (36) were used but were modified slightly. Each sample with 0.02 μg of bromophenol blue was placed in a sample well of a 15% slab gel. Electrophoresis at 35 mA was allowed to proceed just until the dye reached the bottom of the gel. Electrode buffer contained 0.05 M Tris, 0.38 M glycine, and 0.1% sodium dodecyl sulfate.

After electrophoresis the gel slab was dried as described by Maizel (28). Autoradiograms were prepared by exposing Kodak type SB45 medical X-ray film to dried gels for 3 days and were scanned with a Joyce-Loebl microdensitometer. Control experiments showed that autoradiogram film density was proportional both to the exposure time and to the amount of protein layered on the gel. Coat protein was quantified by the method of Casjens and King (7): coat protein peak on the microdensitometer tracing was cut out and weighed as a measure of the relative amount of coat protein present at each sampling time.

Induction and assay of β-galactosidase. Infected or uninfected cells were shifted to fresh medium by filtration (40). β-Galactosidase synthesis was induced at the indicated times by the addition of 0.6 mM isopropyl-β-D-thiogalactopyranoside. Sampling procedures and toluene treatment were as described previously (40). The activity of β-galactosidase was measured by the hydrolysis of 4-methylumbelliferyl-β-D-galactopyranoside. Samples of toluene-treated cells (0.1 ml) were mixed with an equal volume of substrate solution (0.5 mM in water) and incubated for 10 to 20 min at 25°C. The reaction was terminated by adding 0.05 ml of reaction mixture to 1.5 ml of 0.25 M Na_2CO_3. Fluorescence was measured in an Aminco-Bowman spectrophotofluorometer, with the use of an exciting wavelength of 365 nm and an emitting wavelength of 450 nm. Measured fluorescence was converted to nanomoles of 4-methylumbelliferone formed by comparison with standard solutions of 4-methylumbelliferone. Product formation was found to be linear with time over approximately 1 h, and enzyme activity was strictly proportional to the volume of culture assayed. One unit of β-galactosidase activity is defined as that amount catalyzing the formation of 1 nmol of 4-methylumbelliferone per min under these conditions.

Chemicals and radiochemicals. [2-^{14}C]uracil (55 mCi/mmol), [5-^{3}H]uridine (8 Ci/mmol), carrier-free $H_3{}^{32}PO_4$, and rifampin were purchased from Schwarz-Mann, Orangeburg, N.Y., and [^{14}C]proline (240 mCi/mmol) was from New England Nuclear, Boston, Mass. Egg white lysozyme and electrophoretically pure DNase were purchased from Worthington Biocemical Corp., Freehold, N.J.; chloramphenicol, from Calbiochem, Los Angeles, Calif.; and isopropyl-β-D-thiogalactopyranoside and 4-methylumbelliferyl-β-D-galactopyranoside, from Sigma Chemical Co., St. Louis, Mo.

RESULTS

Production of progeny phage after shift-down. When *E. coli* was infected with Qβ and subjected to energy source shift-down 20 min later, the appearance of progeny phage was in-

hibited (Fig. 1). Replicase formation is substantially complete in 20 min of infection (26); thus, we have avoided possible complications due to an effect of shift-down on the synthesis of replicase. Cells were lysed enzymatically, so the sum of intracellular and extracellular phage is represented in Fig. 1. The rate of appearance of progeny phage in the period from 25 min to 40 min in the down-shifted culture was about fourfold lower than in the control culture which was treated in the same manner as the down-shifted culture but resuspended in fresh glucose medium.

Phage RNA replication after shift-down. The use of rifampin allows the direct measurement of phage RNA replication in infected cells (14). Figure 2 shows the rate of incorporation of [^{14}C]uracil into acid-precipitable material in infected cells as a function of the fraction of cells infected [$1 - P(0)$, calculated from the known multiplicity of infection by the Poisson distribution]. It is apparent that the rate of [^{14}C]uracil incorporation was proportional to the number of infected cells. These data provide assurance that the procedure of correcting uptake data in infected cells by subtracting the uptake into uninfected cells is valid. The residual rate of [^{14}C]uracil incorporation in uninfected cells after 10 min of rifampin treatment was low, usually less than 15% of the maximum incorporation rate in infected rifampin-treated cells.

The highest rates shown in Fig. 2 were obtained at a multiplicity of 5 [$1 - P(0) = 0.99$]. At a higher multiplicity (up to 30) the rate of incorporation did not increase further. This is consistent with previous observations (3) that an *E. coli* cell can be penetrated by only one, or at most a small number, of phage RNA molecules. In any case, if more than one phage RNA molecule does penetrate a bacterium, it does not result in an increased rate of incorporation of isotope into phage RNA.

The kinetics of incorporation of [^{14}C]uracil into phage RNA in rifampin-treated cells are shown in Fig. 3. The rate of incorporation in infected down-shifted cells was about fivefold lower than in infected cells shifted to fresh glucose medium. Addition of glucose (45 min) restored the rate of incorporation to a rate nearly that found in exponentially growing cells, even after the addition of chloramphenicol (42 min). This implies that the rate of RNA labeling in down-shifted cells is not limited by the synthesis of any protein necessary for the replication process.

To determine whether this reduced rate of incorporation was due to a lower specific activity of the UTP pool in down-shifted cells, we meas-

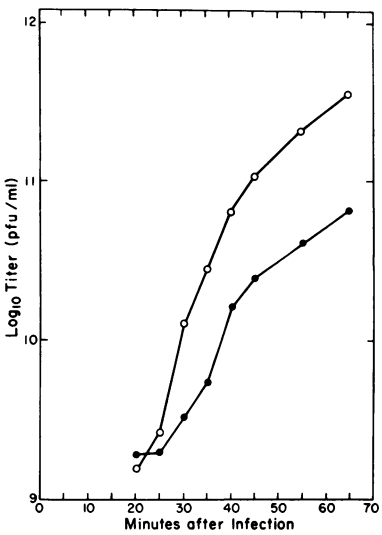

FIG. 1. *Production of Qβ in cultures subjected to a shift-down 20 min after infection. A culture growing in minimal medium was infected with Qβ and 20 min after infection was shifted, part to succinate medium* (●) *and part to fresh glucose medium* (○). *Total phage production (given as PFU/ml) was measured as described in Materials and Methods.*

ured the rate of uptake of [^{14}C]uracil into the UTP pool in cells treated in the same manner as those in the experiment shown in Fig. 3. The rise in the specific activity of the UTP pool following addition of [^{14}C]uracil was monitored in bacteria that had been cultured with [^{3}H]uridine long enough to ensure equilibration of the uridine with the intracellular UTP pool. The ^{3}H radioactivity in extracted UTP was then proportional to the molar amount of cellular UTP at all sampling times, and the ratio of ^{14}C to ^{3}H radioactivity in UTP was proportional to the specific activity of the UTP pool. Figure 4 shows that the uptake of uracil into the UTP pool was inhibited considerably in the down-shifted culture as compared to the control (glucose) culture.

Using these specific activity measurements and the data on the labeling of phage RNA with [^{14}C]uracil (Fig. 3), we calculated the rate of phage RNA replication in down-shifted cells relative to that in growing cells by the method of Lazzarini and Dahlberg (25), with slight modifications. This calculation is illustrated in Table 1. First, the overall rate of [^{14}C]uracil incorporation was corrected for the amount of isotope entering phage RNA in CMP residues to determine the rate of incorporation of isotope into UMP residues only (column 7). Dividing this by the relative specific activity of the UTP pool (column 4) gave a "corrected" rate of incorpo-

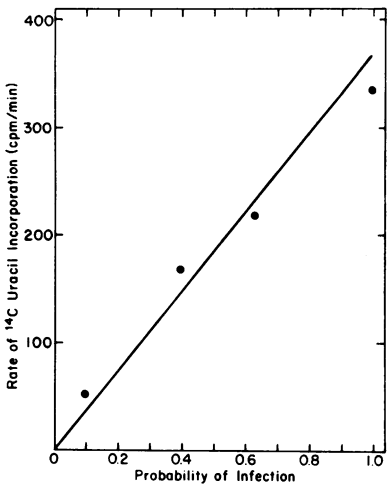

FIG. 2. *Rate of RNA labeling as a function of probability of infection. Cells growing in glucose medium were infected at varying multiplicities. Cultures received rifampin (200 μg/ml) at 15 min after infection and [^{14}C]uracil (1 μCi/ml) at 25 min after infection. Isotope incorporation was measured at 5-min intervals for 25 min, and the rate of incorporation was determined after correcting for residual incorporation into uninfected, rifampin-treated cells as described in Materials and Methods. Multiplicities used were 0.1, 0.5, 1, and 5 PFU/bacterium to give, respectively, 0.10, 0.39, 0.63, and 0.99 probability of infection [1 − P(0) by the Poisson distribution].*

ration (column 8) which was proportional to the mass of UTP incorporated into phage RNA per minute. The final column of Table 1 shows the rate of phage RNA replication in down-shifted cells relative to that in growing cells during each time interval.

The rate of phage RNA replication in down-shifted cells did not differ significantly from that in growing cells. Thus, the in vivo activity of replicase apparently was unaffected by the shift-down. Furthermore, it seems that the reduced rate of incorporation of [^{14}C]uracil (Fig. 3) was due solely to a reduced rate of entry of uracil into the UTP and CTP pools.

The rate of phage RNA replication decreased as early as 35 min after infection both in exponentially growing cultures and in down-shifted cultures (Table 1, column 8). Watson and Yamazaki (39) found that the rate of phage R17 RNA replication in infected growing cells decreased after 24 min of infection and noted that the decrease was paralleled by a decrease in the size of the ATP pool. However, as will be shown, the decreasing rate of RNA replication that we observed does not correlate with a drop in the size of the ATP pool since the size of the ATP pool remained nearly constant at least until 60 min after infection (Table 4).

In both these experiments and in the experiments of Watson and Yamazaki (39), cultures were treated with rifampin, which has been reported to inhibit RNA replication during infection by some RNA phages (8, 29). However, Engelberg et al. (9) showed that rifampin inhibits neither the conversion of Qβ parental RNA into double-stranded form nor the disappearance of parental RNA from that form, and they concluded that Qβ RNA replication is unaffected by the drug. Thus, the decreasing rate of RNA replication that we observed probably is not an artifact of rifampin treatment but may reflect the normal course of RNA replication during infection.

The fact that the total rate of phage RNA synthesis was about the same in the exponential and down-shifted cultures does not necessarily indicate that the amount of RNA available for translation of phage proteins is the same in both cultures. During the course of the infectious cycle, some of the newly formed phage RNA will be encapsulated to form virions and some will be sequestered in the double-stranded intermediates of replication (10).

To determine the distribution of phage RNA

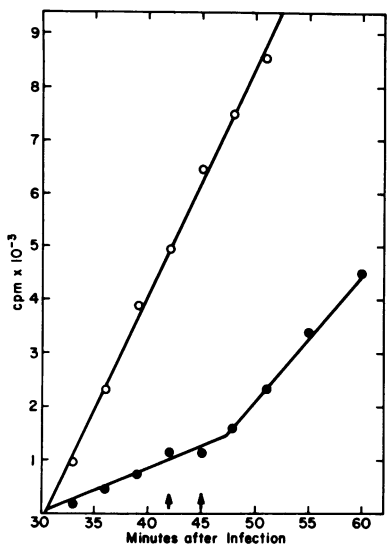

FIG. 3. *Effect of shift-down and shift-up on the kinetics of isotope incorporation into phage RNA. Twenty minutes after infection a culture was shifted, part to succinate medium (●) and part to fresh glucose medium (○), both containing rifampin (200 μg/ml). Cultures received [^{14}C]uracil (1 μCi/ml) 30 min after infection. The down-shifted culture received chloramphenicol (100 μg/ml) at 42 min and 10 mM glucose at 45 min after infection (times indicated by arrows). Acid-insoluble radioactivity was determined in duplicate samples (0.05 ml), and the data were corrected for residual incorporation into uninfected cells.*

between these various forms, we compared the fraction of RNase-resistant radioactivity in the RNA from growing and down-shifted cells. The

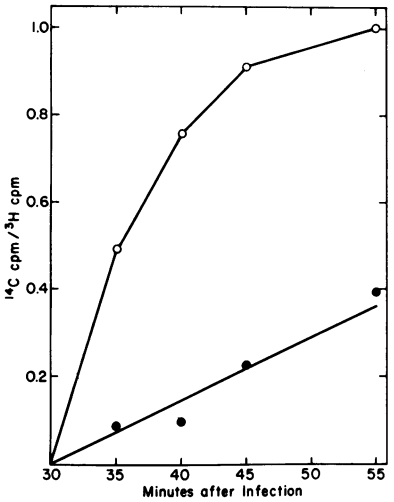

FIG. 4. *Entry of exogenous uracil into the cellular UTP pool. A culture received 2.5 μCi (0.31 nmol) of [^{3}H]uridine per ml at 60 min before infection. Twenty minutes after infection the culture was shifted, part to succinate medium (●) and part to fresh glucose medium (○), both containing rifampin (200 μg/ml). Cultures received [^{14}C]uracil (1 μCi/ml) 30 min after infection. Nucleotides were extracted, concentrated, and separated as described in Materials and Methods. The relative specific activity of the UTP pool at each sampling time is expressed as ^{14}C cpm/^{3}H cpm.*

double-stranded forms of phage RNA are resistant to RNase digestion (10), and the RNA in infectious virions is protected from digestion by the capsid protein (1, 18). Sucrose gradient analysis (data not shown) of an RNase-treated lysate from glucose-grown cells showed that 35% of the radioactivity appeared in a peak corresponding to intact virions (84S) and 47% of the radioactivity sedimented at <25S, presumably representing the double-stranded forms of phage RNA. No peak corresponding to ribosomes (70S) was present in the RNase-treated lysates, and extracts of uninfected cells lacked the 84S virion peak.

The data in Table 2 show that the fraction of RNase-resistant radioactivity was the same (19 to 20%) in the growing and down-shifted cultures. It follows that the fraction of single-stranded phage RNA also was the same in both cultures. Since down-shifted cells and growing cells contained both the same proportion of these different species of phage RNA and the same total amount of phage RNA, it is likely that the amount of RNA available for translation of phage proteins was the same in both cultures.

Phage coat protein synthesis after shift-down. From the results presented above, we concluded that the reduced production of progeny phage following shift-down was not due to an inhibition of phage RNA replication. Furthermore, the observation that the rate of phage RNA replication was unaffected by the shift-

TABLE 1. *Relative rate of phage RNA replication*[a]

Medium	Rate of Incorporation of [^{14}C]uracil[b] (cpm/0.05 ml/min)	Time interval (min after infection)	Mean relative sp act[c] (^{14}C cpm/^{3}H cpm)		Fraction of ^{14}C incorporated into UMP residues[d]	Rate of incorporation of ^{14}C into UMP residues[e] (cpm/0.05 ml/min)	Corrected rate of incorporation[f] (cpm/0.05 ml/min)	Relative rate of RNA replication[g]
			UTP	CTP				
Glucose	403	30–35	0.25	0.06	0.83	335	1340	1.0
		35–40	0.63	0.17	0.82	331	530	1.0
		40–45	0.84	0.24	0.81	327	390	1.0
Succinate	84	30–35	0.05	0.02	0.75	63	1270	0.9
		35–40	0.09	0.06	0.64	54	600	1.1
		40–45	0.16	0.10	0.66	56	350	0.9

[a] Calculated by the method of Lazzarini and Dahlberg (25), slightly modified.

[b] Using data from Fig. 3, determined from least-squares analysis of either all data for the glucose culture or all data points between 33 and 55 min after infection, inclusive, for the succinate culture.

[c] Using data from Fig. 4 and data for the kinetics of [^{14}C]uracil incorporation into CTP pools from the same experiment but not shown calculated as the mean relative specific activity for each time interval: $[(XTP_{t_2} - XTP_{t_1})/2] + XTP_{t_1}$.

[d] Calculated as 0.295 UTP specific activity/(0.295 UTP specific activity + 0.247 CTP specific activity), calculated with the mean relative specific activity of the CTP and UTP pools and the relative abundance of CMP and UMP residues in Qβ plus strands (24.7% and 29.5%, respectively [32]).

[e] Rate of incorporation of [^{14}C]uracil × fraction of ^{14}C incorporated into UMP.

[f] Rate of incorporation of ^{14}C into UMP residues/mean relative specific activity of UTP pool.

[g] Corrected rate of incorporation/corrected rate of incorporation in glucose medium during the same time interval.

TABLE 2. *RNase-resistant phage RNA*[a]

Growth condition	Radioactivity in lysate[b]		Percent RNase-resistant[c]
	−RNase	+RNase	
Glucose, infected	69,445	16,145	19.2
Glucose, uninfected	4,255	3,630	
Succinate, infected	10,310	2,610	19.7
Succinate, uninfected	755	730	

[a] Twenty minutes after infection a culture was shifted, part to succinate medium and part to fresh glucose medium, both containing rifampin (200 μg/ml). Cultures received [^{14}C]uracil (1 μCi/ml) 30 min after infection. Lysates were prepared 45 min after infection and treated with RNase. An uninfected control culture was treated in an identical manner.

[b] Given as counts per minute per milliliter of culture.

[c] The ratio of RNase-resistant radioactivity (infected minus uninfected) to total radioactivity (infected minus uninfected).

down indicated that down-shifted and growing cells contained equivalent amounts of RNA. This allowed us to measure the effect of shift-down on the translation of phage RNA in vivo without possible complications due to an effect of shift-down on the rate of formation of that RNA.

We measured the rate of Qβ coat protein synthesis in cells subjected to a shift-down and treated with rifampin 20 min after infection. Proteins labeled with [^{14}C]proline were separated by polyacrylamide slab gel electrophoresis in the presence of sodium dodecyl sulfate. A typical microdensitometer tracing of the autoradiogram of a dried gel is shown in Fig. 5. The predominant peak, with a molecular weight of about 14,000, was found in infected cells and was presumed to represent coat protein (23).

Autoradiogram film density, as determined by the area under the coat protein peak on microdensitometer tracings, was found to be proportional to the amount of protein layered on the gel; therefore, to measure the relative amount of coat synthesized during different intervals following a shift-down, we cut out the coat protein peaks on tracings and weighed them (Table 3).

It can be seen that over the period from 30 to 45 min after infection, during which the number of viable phage particles in the culture increased approximately 10-fold (see Fig. 1), the rate of coat protein synthesis in the culture shifted to succinate medium was approximately 20% of that in the culture shifted to fresh glucose medium. The reduction in the rate of coat protein synthesis in down-shifted cells was about the same as the reduction in the rate of appearance of viable progeny phage (Fig. 1). In view of the fact that the rate of RNA replication in both cultures was the same over this period (Table 1), it seems probable that the lower rate of production of viable phage in the down-shifted cultures was a direct result of the reduced rate of coat protein synthesis.

Polypeptide chain growth rates. To determine whether the rate of polypeptide chain growth was affected by the shift-down, we measured the lag time for β-galactosidase induction in both uninfected and infected cells in glucose medium, and in down-shifted, infected cells. As shown in Fig. 6, a plot of the square root of the increment in enzyme activity against time gave a straight line. The intercept of this line on the time axis represents the time required to synthesize a single β-galactosidase polypeptide (35), and is thus a composite of the times required for derepression, transcriptional completion, and translational completion. In uninfected cells in glucose medium, this completion time was 95 s,

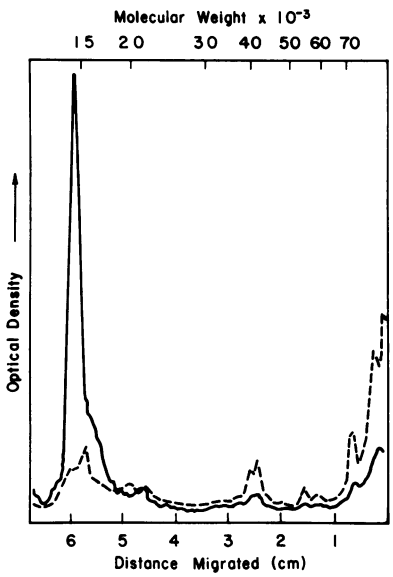

FIG. 5. *Microdensitometer tracing of an autoradiogram of a dried slab gel. Cells were treated as described in Materials and Methods. Labeled proteins from down-shifted cells harvested 40 min after infection were separated by polyacrylamide slab gel electrophoresis in the presence of sodium dodecyl sulfate. The autoradiogram of the dried gel slab was traced with a Joyce Loebl microdensitometer (solid line). Proteins from an uninfected control culture were separated and visualized in the same manner (dashed line). The molecular weight scale on the abscissa was determined by electrophoresis of proteins of known molecular weight on the same gel slab. The standards used and their molecular weights were bovine serum albumin (68,000), ovalbumin (43,000), pepsin (35,000), chymotrypsinogen (25,700), and myoglobin (17,000).*

TABLE 3. *Relative rate of coat protein synthesis*[a]

Time interval (min after infection)	Culture medium	Coat protein (mg)[b]	Relative rate of coat protein synthesis[c]
30–35	Glucose	240	1.00
	Succinate	86	0.36
30–40	Glucose	670	1.00
	Succinate	150	0.22
30–45	Glucose	1,200	1.00
	Succinate	220	0.18

[a] See Materials and Methods.

[b] Weight of the coat peak from microdensitometer tracings of autoradiograms of dried gels.

[c] Coat protein synthesized during each time interval/coat protein synthesized in glucose medium during the same time interval.

corresponding to a polypeptide chain growth rate of 12 amino acids per second. In infected cells, the completion time was 105 s, corresponding to a chain growth rate of 11 amino acids per second. Most significantly, the completion time was the same for infected cells in glucose medium and at 20 min after a shift to succinate medium. These conditions are identical to those under which we measured the synthesis of Qβ coat protein, except for the necessary omission of rifampin during β-galactosidase induction. We therefore conclude that the polypeptide chain growth rate is unaffected by the shift-down in infected cells. We have not further investigated the small increase in completion time which appears to accompany Qβ infection.

Nucleotide levels in down-shifted cells. In order to determine whether the reduced rate of phage coat protein synthesis following shift-down correlates with changes in nucleotide pool levels, ^{32}P-labeled nucleotides were extracted and resolved by thin-layer chromatography. Table 4 shows that the ATP pool size was nearly constant after shift-down, suggesting that the general level of energy was not a limiting factor. On the other hand, by 40 min after shift-down the GTP pool level dropped to 60% of the level found in growing cells before the shift-down. There was only a small accumulation of ppGpp in these rifampin-treated cells (Table 4). In the absence of rifampin, we saw a five- to sevenfold increase in ppGpp (data not shown), but, since it did not accumulate significantly in cells during these experiments, ppGpp apparently was not the primary effector of the inhibited synthesis of coat protein that we observed.

DISCUSSION

We have sought to determine whether the translation of bacteriophage Qβ RNA in down-shifted *E. coli* is inhibited in a manner similar to that previously observed for total *E. coli* protein and for β-galactosidase (40). In the present experiments, infected cells were down-shifted after the synthesis of phage replicase protein was substantially complete, to avoid complications due to an effect of shift-down on replicase synthesis.

Although the rate of incorporation of exogenous radioactive uracil into phage RNA was greatly reduced in the down-shifted culture (Fig. 3), as was also observed by Friesen (11), this only represented a reduced rate of entry of exogenous uracil into the intracellular UTP pool (Fig. 4). After appropriate correction for the relative specific activities of the UTP pools (Table 1), the rate of Qβ RNA synthesis was seen to be the same in the exponential (glucose) and down-shifted (succinate) cultures. Thus, although the inhibition of rRNA synthesis following an energy source shift-down has been carefully documented (16), the claim (11) of similar control of phage RNA replication does not seem to be valid.

Since the rate of phage RNA replication and the fraction of phage RNA in RNase-resistant forms was the same in the two cultures (Table 2), we conclude that the amount of single-stranded phage-specific RNA available for translation of phage proteins was the same. Nevertheless, the rate of synthesis of the Qβ coat protein was reduced by three- to fivefold in the down-shifted cultures (Table 3). This is approximately the same degree of inhibition as was observed for total *E. coli* protein (34, 40).

Considering that the amount of translatable

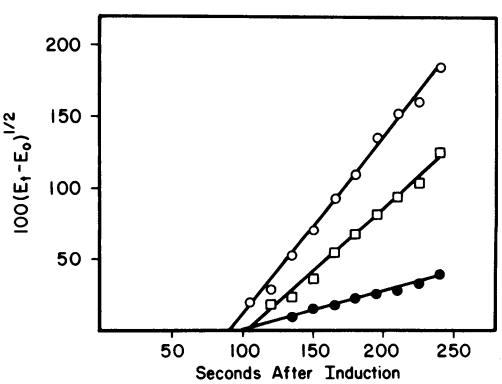

FIG. 6. *Induction kinetics of β-galactosidase in uninfected and infected cells. Uninfected cells were shifted to fresh glucose medium and induced immediately (○). Infected cells were shifted at 20 min after infection into fresh glucose medium and induced immediately (□) or into succinate medium and induced 20 min after the shift (●). Initial enzyme activity (E_o) was 0.2 to 0.22 U/10^9 cells. The lines shown are least-squares fits to the data points.*

TABLE 4. *Nucleotide pool levels*[a]

Time after shift-down (min)	Culture medium	ATP		GTP		ppGpp	
		Amt[b]	Relative pool size[c]	Amt[b]	Relative pool size[c]	Amt[b]	Relative pool size[c]
−10	Glucose	3.0	1.0	2.0	1.0	0.08	1.0
10	Glucose	3.9	1.3	2.3	1.2	0.06	0.8
	Succinate	3.8	1.3	1.8	0.9	0.11	1.3
20	Glucose	4.2	1.4	2.7	1.4	0.07	0.9
	Succinate	3.2	1.1	1.3	0.7	0.10	1.3
30	Glucose	4.8	1.6	2.7	1.4	0.06	0.8
	Succinate	3.4	1.1	1.4	0.7	0.11	1.4
40	Glucose	4.3	1.4	2.2	1.1	0.07	0.9
	Succinate	3.3	1.1	1.2	0.6	0.11	1.4

[a] Cells cultured in medium containing $^{32}P_i$ were infected and 20 min later were shifted, part to succinate medium and part to fresh glucose medium, both containing rifampin (200 μg/ml). At the indicated times nucleotides were extracted and separated, and levels were determined as described in Materials and Methods.

[b] Nanomoles per optical density unit.

[c] Ratio of nucleotide level to the level before shift-down.

Qβ RNA was no different in the exponential and down-shifted cultures, this inhibition of protein synthesis might arise through a change in either the rate of polypeptide chain initiation or in the rate of polypeptide chain elongation.

The data of Fig. 6 show that, although there was a small increase (~10%) in the β-galactosidase completion time attendant upon Qβ infection, the polypeptide chain propagation rate in infected cells was unaffected by a shift from glucose medium to succinate medium. Westover and Jacobson (40) also found no change in the polypeptide chain propagation rate in uninfected cells of another *E. coli* strain. On the other hand, Johnsen et al. (22) reported that a shift-down produced by growth inhibition with α-methylglucoside leads to a decreased polypeptide chain growth rate, especially in strains carrying the *relA* allele. This discrepancy suggests either that *E. coli* protein synthesis responds differently to various shift-down regimens or that different responses may be shown by different *E. coli* strains. This point remains to be clarified.

Finally, we note that the rate of protein accumulation is not likely to be markedly influenced by protein turnover, as Westover and Jacobson (40) have shown that both the rate and extent of degradation of newly synthesized protein after the shift-down are the same as in exponentially growing cultures.

It therefore seems probable to us that translation of the Qβ coat protein cistron is regulated in the same manner as that of host mRNA's, namely, at the initiation of translation.

These results suggest that the mechanism which acts to inhibit translational initiation after an energy source shift-down is relatively nonspecific. Although Ruscetti and Jacobson (34) speculated that the small residual number of polyribosomes which persist in down-shifted cells might represent the exemption of some mRNA species from control of translational initiation, no such mRNA species has yet been identified.

Westover and Jacobson (40) showed that the degree of inhibition of translational initiation after a shift-down was the same for total protein (presumably translated largely from monocistronic mRNA's) and for the 5′-proximal cistron of a polycistronic mRNA (β-galactosidase). Jacobson and Baldassare (21) examined the accumulated 70S ribosomal mRNA complexes by electron microscopy and showed that the length distribution of the bound mRNA strands was consistent with a normal population of monocistronic mRNA's. Strands long enough to be polycistronic mRNA's were not observed, probably because such very long mRNA's would, on the average, be associated with more than one ribosome (41). Thus, the question of whether the same control was exerted on the translation of internal cistrons of polycistronic mRNA's remained unresolved.

The present data show that the translation of Qβ coat protein from the middle cistron of a tricistronic mRNA is subject to inhibition which quantitatively resembles that exerted on the synthesis of other proteins after shift-down. We conclude from this that the mechanism which regulates translation after an energy source shift-down does not involve any specific role for the 5′ end of the mRNA.

The nature of the effector(s) governing this

inhibition remains unknown. In the present study, we measured the intracellular concentrations of three possible effectors: ATP, GTP, and ppGpp.

Although substantial declines in intracellular ATP pools have been reported following an energy source shift-down (16, 17, 31), our data (Table 4) show that this did not occur under the specific conditions of our experiments. It seems likely that rifampin, by decreasing the expenditure of ATP for cellular RNA synthesis, produces a net expansion of the ATP pool in glucose cultures and prevents a net contraction of the ATP pool in down-shifted cultures (Table 4). The fact that the translation rate decreased even when the ATP pool was thus maintained at a near normal level shows that ATP deficiency cannot be responsible for the decreased rate of translation. Swedes, Sedo, and Atkinson (37) have also argued, from experiments on cells starved simultaneously for glucose and adenine, that the rate of protein synthesis is independent of the absolute size of the ATP pool.

Our measurements also show that the GTP pool declined by about 40% after the shift-down (Table 4). Similar declines in intracellular GTP following shift-down have also been observed by others (16, 42). The present case differs from previous observations in that the contraction of the GTP pool took place in the absence of ppGpp accumulation (see below). This indicates that neither the withdrawal of guanine nucleotides to form ppGpp nor inhibitory effects of ppGpp on guanine nucleotide biosynthesis (15) are contributing to the loss of GTP. Rather, it seems that the contraction of the GTP pool must be a more direct effect of the shift-down.

Although there is nothing in the present data to rule out the possibility that this decline in the GTP pool is causally related to the decreased rate of translation, we regard such rough temporal correlations as insufficient proof of a causal relationship. More detailed kinetic studies of the relationship between the GTP pool and inhibition of translation are now in progress in our laboratory.

A number of kinds of evidence have been interpreted as suggesting that ppGpp, which accumulates significantly in both *relA*$^+$ and *relA* cells after a shift-down (42), might be a physiological regulator of translation. These include both stimulatory and inhibitory effects of ppGpp on in vitro protein synthesis (44), inhibitory effects of ppGpp on in vitro assays for specific components of the translational apparatus (30, 45), and temporal correlations between changes in protein synthesis and changes in cellular ppGpp pools (24). In the present experiments, we found that ppGpp did not accumulate significantly in down-shifted, infected cells treated with rifampin (Table 4). The fact that the translation of Qβ coat protein was nevertheless inhibited indicates that ppGpp is not the primary effector of translational inhibition. This represents the first case in which inhibition of translation after a shift-down has been shown to be independent of ppGpp accumulation.

It should be noted that the failure of these cultures to accumulate ppGpp after an energy-source shift-down was a specific result of the use of rifampin in our experiments, since ppGpp accumulated five- to sevenfold over the basal (glucose culture) level when rifampin was omitted (data not shown). This contrasts with the pattern of ppGpp accumulation found by Watson and Yamazaki (39) in amino acid-starved cultures. They found that rifampin prevented ppGpp accumulation in uninfected but not in infected amino acid-starved cultures, and they concluded that accumulation of ppGpp in rifampin-treated cultures was phage specific. Similarly, Lund and Kjeldgaard (27) showed that rifampin-induced decay of ppGpp does not occur during infection with bacteriophage T7. It was postulated that ppGpp accumulation is dependent on the presence of translatable mRNA and that infection with RNA phages or T7 provides a source of mRNA even in the presence of rifampin.

Our data, however, indicate that the presence of translatable Qβ RNA in down-shifted cells is not sufficient to relieve rifampin inhibition of ppGpp accumulation. This apparent discrepancy may reflect nothing more than the fact that the *relA*-independent accumulation of ppGpp after a shift-down occurs by a different mechanism than the *relA*-dependent accumulation of ppGpp in amino acid-starved cells (5). Alternatively, it may be that quantitative aspects of the mRNA supply and its rate of participation in translation are important.

Many steps in the infectious cycle of RNA phages either directly or indirectly involve the translational machinery of the host bacterium. Not only does the phage RNA have to act as both mRNA and template for RNA replication, but ribosome-associated proteins participate directly in the process of RNA-dependent RNA replication (2, 38). It therefore seems likely that an inhibition of phage RNA translation will have further secondary consequences for phage development. Leschine and Jacobson (Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, K45, p. 144) reported that the appearance of Qβ coat protein and RNA-dependent RNA replicase activity are delayed considerably when a shift-

down is imposed just before, but not just after, Qβ infection. Further study of the consequences of translational inhibition for the early events of phage development may help to delineate the mechanism of translational control.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM-20114 from the National Institutes of Health and by grant G-70-23 from the Life Insurance Medical Research Fund.

We are grateful to Richard Lengyel for skilled technical assistance.

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